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TECHNICAL REPORT NO. LWL-CR-09C71

RAPID DETERMINATION OF HEROIN (MORPHINE) IN URINE

Final Report
Contract No. DAAD05-72-C-0187

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PREFACE

This report contains the results of a 6-month program designed to improve an existing field kit for the detection of morphine in urine. The report covers work conducted over the period 10 February 1972 to 15 August 1972. The project leader was Dr. E. J. Woodhouse, Senior Chemist, who was assisted by Mr. G. W. Webb and Mr. M. Serrone, research technicians.

Approved for:

MIDWEST RESEARCH INSTITUTE

A handwritten signature in dark ink, appearing to read 'H. M. Hubbard', is written over the printed name. The signature is fluid and cursive, with a long, sweeping underline that extends to the right.

H. M. Hubbard, Director
Physical Sciences Division

18 October 1972

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SUMMARY

This report details work conducted on a project designed to improve the sensitivity of a field test kit for detecting morphine in urine. Color reagents, extraction parameters and techniques were evaluated to improve the sensitivity to less than 1 µg/ml morphine in urine. Final evaluation of the redesigned field kit using spiked urine indicated the project was successful; however, evaluation with user urine was inconclusive due to the shortage of a supply of fresh user urine.

I. INTRODUCTION

The presently available field test kit for morphine in urine is not of sufficient sensitivity to be of practical use to the Armed Forces. This report, the Final Report of a 6-month program to improve the present kit, details the objectives, accomplishments and results, problems encountered and solutions, conclusions, and recommendations resulting from this program.

II. OBJECTIVES

The objective of this project was to achieve a suitable procedure for the existing morphine test kit by which the sensitivity could be increased from about 4 µg morphine per milliliter urine to concentrations of morphine in urine of less than 1 µg/ml.

The presently available kit consists of solvent and buffer for the liquid extraction of morphine from urine followed by separation of the liquid phases by gravity and transposition of organic solvent by pipet to a small tube containing color reagent which yielded a purple color if morphine was present.

In order to accomplish the above objective, a research program was designed to include the following steps:

1. Evaluation of the literature available on morphine detection and chemistry.
2. Evaluation of indicators or reagents for the colorimetric determination of morphine in urine.
3. Evaluation of the extraction process to permit the detection of morphine in urine in quantities less than 1 µg/ml.
4. Evaluation and analysis of the data generated to determine if the existing technique can be made to generate the required sensitivity.
5. Actual testing of reagents and techniques resulting from the above evaluation.
6. Fabrication of reagents for testing at a government facility.

The accomplishments and results of this program are detailed in the next section of this report.

III. ACCOMPLISHMENTS AND RESULTS

The accomplishments and results of this research program are presented in the following order:

1. Evaluation of the literature
2. Evaluation of color reagents
3. Evaluation of the extraction process
4. Evaluation of data generated
5. Testing of reagents and techniques
6. Fabrication of reagents

A. Evaluation of the Literature

A survey of scientific literature on the color reactions, chemistry, extraction and solubility characteristics of morphine was conducted. Citations from 1940 to the present were retrieved using Chemical Abstracts and Chemical Titles. Many of the articles retrieved refer to earlier work which was retrieved also. A listing of the literature retrieved is presented in Section VII of this report, Citations Nos. 1-46.

The literature reviewed indicated mainly the classical color reactions of oxidants in strong acid, or organic compounds such as aldehydes in strong acid. The majority of color reagents are summarized in two texts. "The Chemistry of the Morphine Alkaloids," Clarendon Press, Oxford, 1954 and "Narcotic Drugs," Plenum Press, New York, 1971. Most of the color reactions known are those for spot tests in which the concentration of morphine is relatively high. Those which showed promise as color reactions for a solution test in microgram quantities are listed in Table I.

B. Evaluation of Color Reagents

The evaluation of color reagents for morphine was conducted in two phases:

1. Investigation of the sensitivity of the color reagent to morphine.

2. Investigation of possible interferences by other drugs.

These phases, and the results obtained are described below:

1. Investigation of the sensitivity of the color reagent: All the color reagents found in the literature and cited in Table I were examined

for their sensitivity in the color reaction with morphine. Other reagents and modifications of the reagents in Table I were also examined. All of these reagents were chosen for their potential suitability for a urine test similar in design to the present kit. Criteria included potential color change, ease of performance, and compatibility with organic solvents.

The evaluation of the sensitivity of the color reagents was conducted by reproducing the final step of the field kit procedure, i.e., mixing the color reagent in a small vial with a solution of morphine in organic solvent. The solvent was isopropyl alcohol/chloroform 1:3, as originally used in the present kit. The solvent was equilibrated by shaking with buffer (125 ml solvent + 250 ml water containing 37 g of a 1:1:1 mixture of sodium carbonate/sodium borate/sodium bromide) or sensitivity was lost. This buffer equilibration corresponds to the treatment the solvent receives in an actual urine test as modified by Mr. Ralph Allen of LWL.

Initial evaluation of the color reagents was conducted using 500 µg/ml solutions of morphine in buffer treated solvent. Controls were run in each case using plain buffer treated solvent. If the color reagent showed promise then it was tested with more dilute solutions of morphine in buffer treated solvent.

Table II lists the results of the evaluation for 113 color reagents. Color reactions for test solutions and controls are listed for experiments with solutions containing 500 µg/ml morphine. If the results showed promise, experimental results are also listed for 5 µg/ml and 0.5 µg/ml solutions of morphine.

The results in Table II indicate that color Reagent No. 14, sodium molybdate, 10% in concentrated sulfuric acid, provided the most sensitive color test for morphine using the system as described in the above work. Reagent No. 28 was also very sensitive but suffered from an instability not found with Reagent No. 14.

With a concentration of 5 µg morphine in 1 ml of solvent, Reagent No. 14 gave a medium purple/pink color, whereas the original LWL reagent gave a lavender color not as well pronounced. In an actual test with urine, this would represent a concentration of 0.5 µg/ml morphine in urine if 10-ml urine were used and extraction efficiency and solvent separation were 100%.

Reagent No. 14 yielded a color test more sensitive than the original LWL color reagent. The obscuring blue color formed less quickly with Reagent No. 14 than with the original LWL reagent.

2. Investigation of possible interferences: Twenty-one drugs were tested to see if they interfered with the color test for morphine when Reagent No. 14 was used. The tests were conducted as follows (shown on p. 24):

To 10 ml of fresh plain urine (male), drug was added to spike the urine to levels of (a) 10 µg/ml, and (b) 5 µg/ml. Buffer (1/2 g of sodium carbonate/sodium borate/sodium bromide 1:1:1) was added and the urine shaken in the plastic tubes provided in the present LWL kit. After solution of the buffer, 5 ml of original LWL solvent was added and the tube gently shaken for 30 sec. The mixture was then poured into a phase-separating filter paper and the organic solvent allowed to drip into a vial containing 1 ml of the color reagent. After 1 ml of the solvent had entered the vial, it was shaken vigorously for 1 or 2 sec. and color formation noted. The results for tests with 21 drugs are noted in Table III.

Of the drugs tested, only paragoric and heroin gave positive tests which were similar to morphine. This is to be expected since paragoric contains morphine and heroin is very similar to morphine. We do not feel this in any way limits the usefulness of the test since heroin never appears in the urine and there should be a record if paragoric has been consumed legally.

C. Evaluation of the Extraction Process

The sensitivity of the system as used in the field kit is directly dependent on the efficiency of the solvent extraction process. Seventy solvents and combinations of solvents were evaluated for extraction efficiency. The evaluation consisted of running actual tests using urine spiked with morphine as in the evaluation of color reagents. The colors produced by color Reagent No. 14 were noted for tests using each of the 70 different solvents. The solvents were chosen for extraction efficiency and compatibility with the color reagent.

Table IV lists the results of these tests.

Controls were run in each case. The best solvents appeared to be the isopropanol/chloroform mixtures similar to the original solvent. A close examination of isopropanol/chloroform ratios revealed that 30% isopropanol in chloroform (the original LWL solvent) was optimum for the color reagent employed. Variation of this composition in either direction detracts from the extraction efficiency.

Since the extraction of morphine depends on the pH of the aqueous medium, the variation of extraction efficiency versus the pH of the medium was investigated. Color reactions were used as a measure of extraction efficiency. Color reactions were conducted using improved LWL buffer (borate/carbonate/bromide, 1:1:1) and sodium molybdate color reagent. The pH of the aqueous medium was varied from 6 to 11 using acid or base. The most intense colors were observed when the pH was 9.3 in the aqueous phase before extraction.

Components of the improved LWL buffer were investigated for their individual characteristics. One and one-half grams of improved LWL buffer (the amount used in a regular test) contains 1/2 g each of sodium borate, sodium carbonate and sodium bromide. One-half gram of borate alone brought the pH to 9.1, one-half gram of carbonate alone brought the pH to 11.7. Bromide itself did not significantly alter the pH. The intensity of the color in a typical test was increased if the borate alone was used at a level of 1/2 of 1 g. Thus, a test run with improved LWL buffer (1-1/2 g) gave pH of 9.9; a test run with sodium borate (1/2 g) gave pH of 9.1, and a more intense color.

Further tests were run to find a material to complement the borate in the buffer and produce an even more intense color test. The following materials (1 g) were added to borate (1/2 g):

Sodium bromide	Potassium chloride
Sodium chloride	Sodium phosphate (dibasic)
Potassium nitrate	Sodium phosphate (tribasic)
Calcium chloride	Sodium polyphosphate
Potassium bromide	Sodium metasilicate
Sodium nitrate	Sodium orthosilicate
Sodium sulfate	Sodium perborate

Of all the above materials, sodium chloride was the most effective, resulting in a more intense color than the other materials.

Since urine may often be quite acidic, we compared the test using improved LWL buffer and borate/chloride (1:2) using urine originally at pH 4.0. The improved LWL buffer changed the pH to 9.6, the borate/chloride changed the pH to 8.3. Tests were run many times, and in each case the borate/chloride gave a more distinguishable purple color which was not obscured by blue as soon as with the improved LWL buffer.

A final test comparison was run using the original LWL buffer, the improved LWL buffer and the borate/chloride buffer. Concentration of morphine in the urine was gradually dropped to determine the sensitivity limit. The following table indicates the results which were duplicated many times.

<u>Urine Solution</u>	<u>Buffer</u>		
	<u>Original LWL</u>	<u>Improved LWL</u>	<u>Borate/Chloride (1:2)</u>
7 µg/ml morphine	Purple	Purple	Purple
3 µg/ml morphine	Medium Purple	Medium Purple	Medium Purple
2 µg/ml morphine	Weak Purple	Weak Purple	Light Purple
1 µg/ml morphine	Negative	Extremely Weak Purple Flash	Weak Purple

In the above experiments, sodium molybdate, 10% in concentrated sulfuric acid (Reagent No. 14), was used as the color reagent.

D. Evaluation of the Data Generated

The data generated in the program indicated that the original RPC test kit could be improved in sensitivity by:

1. Employing sodium molybdate (10% in concentrated sulfuric acid) as the color reagent.
2. Retaining the original solvent (30% isopropanol in chloroform).
3. Employing 1-1/2 g of sodium borate/sodium chloride (1:2) as buffer.

The sensitivity of the test was at least 1 µg morphine per milliliter urine. In all cases, the test was modified as suggested by LWL, i.e., the use of phase separating filter paper has replaced the centrifuge and pipet stage of the operation.

Repeated tests using fresh urine spiked with morphine and the above modifications indicated that a sensitivity level of less than 1 µg/ml urine was achievable.

In order to evaluate fully the sensitivity of the modified test, experiments involving both spiked and user urine samples were conducted as described in the next section.

E. Testing of Reagents and Techniques

The reagents and techniques evaluated in the program so far were evaluated for effectiveness using both spiked urine and user urine from morphine addicts. The technique employed was as follows:

Fifteen milliliters of urine (spiked with morphine sulfate or from a morphine addict) was placed in a plastic centrifuge tube; 1.5 g of buffer (borate/chloride, 1:2) was then added and the urine shaken to dissolve the solids; 5 ml of solvent (30% isopropanol in chloroform) was then added to the mixture and the tube shaken gently by tilting 12 times. The mixture was then poured through a phase-separating filter paper and the organic phase (1 ml) allowed to filter through the paper into a small vial containing 1 ml of color reagent (10% sodium molybdate in concentrated sulfuric acid). The small vial was closed, shaken violently for about 2 sec and the color in the lower layer observed immediately. A purple color indicated a positive.

Table V lists results of tests conducted on fresh urine. Controls were run after every test and were negative in all cases.

The results on the spiked samples indicate that 100% of the samples containing 3 µg/ml, 2 µg/ml, and 1-1/2 µg/ml gave positives. Ninety-four percent of those at 1 µg/ml, 32% of those at 2/3 µg/ml and 39% of those at 1/2 µg/ml gave positives.

Table VI lists results of blind tests conducted on user urine obtained from NIMH, Lexington, Kentucky. The urine was assayed fluorimetrically for morphine content by the method of Santinga and Goldbaum.^{1/} Table VI summarizes the quantitative morphine assays and compares them with the results of the field color test run on the same samples.

There were eight observations (four observers, two runs) on each user urine sample tested by the field color test. The results in Table VI indicate the number of positive observations/total observations. Of 188 observations on blank controls; 183 of those observations were negative.

The results with the user samples were inconsistent. Positives were obtained with all the pool samples from 1.07 to 2.35 µg/ml. These pools were mixtures of urine from different patients. The age of the pool samples is not known. The patient samples gave very poor results. Of those samples containing more than 1.00 µg/ml morphine in urine, only 83 positive observations were recorded out of 184 total observations.

F. Fabrication of Reagents

Reagents consisting of 100 vials of color reagent (sodium molybdate, 10% in concentrated sulfuric acid, 1 ml) and 110 vials of buffer (sodium borate/sodium chloride 1:2, 1.5 g) have been prepared and shipped to the government for evaluation at a government facility.

IV. PROBLEMS ENCOUNTERED AND SOLUTIONS

Only one major problem was encountered in this study and this was the acquisition of suitable user urine for the final evaluation of the new kit materials. The user urine obtained was over 3 months old and did not yield satisfactory results with the test kit. This is discussed further in the next section. The solution to this problem is to acquire fresh user urine on the spot at NIMH Lexington, Kentucky, or elsewhere.

^{1/} Santinga, P. (reporting on Goldbaum's method), Fluorescence News, 6(3), 1 (1971).

V. CONCLUSIONS

The conclusions from this program are:

1. Using spiked fresh urine, the new reagents and techniques can produce a kit capable of detecting morphine down to and below 1 µg morphine/ml urine.

The new reagents are:

Color reagent, 1 ml of 10% sodium molybdate in concentrated sulfuric acid

(This replaces the original color reagent (1 ml of 10% ammonium molybdate) in concentrated sulfuric acid.)

Buffer, 1.5 g of Sodium Borate/Sodium Chloride (1:2)

(This replaces the original buffer.)

The solvent remains as the original LWL solvent (30% isopropanol in chloroform).

The new techniques are: The use of phase-separating filter paper (Whatman 1 PS, 11.0 cm diameter) as a means of separating the extraction phases. This replaces the pipet method used in the original LWL kit.

2. Using 3-month old user urine, the new reagents and techniques produce a detection limit which is difficult to ascertain, but is certainly not below 1 µg/ml. The sensitivity limit seems inconsistent and poor when compared with the spiked fresh urine.

We feel that the poor consistency and low sensitivity of the test when conducted with the user urine is due to the age of the urine (3 months). Previous work on the evaluation of the original LWL test indicated that it was capable of detecting 4 µg/ml morphine in urine when fresh spiked urine was used. This was also the level of sensitivity when fresh user urine was used. The test reagents have been formulated to work with fresh urine and we do not feel that they have been justly evaluated with the urine provided by NIMH, Lexington, Kentucky.

VI. RECOMMENDATIONS

In view of the results obtained with the new kit reagents and materials, the following recommendation is offered:

The new reagents and techniques should be evaluated by a blind study using fresh user urine from morphine addicts or users. To be certain of the increased usefulness of the new kit over the original kit, it is also suggested that the original kit and LWL's modified original kit (change in buffer and use of phase separating paper) also be evaluated using the same batch of user urine samples.

We would also recommend a larger blind study using spiked urine with the original LWL kit, LWL's modified kit and the new kit.

TABLE I

COLOR REAGENTS FOR EVALUATION IN THE MORPHINE TEST

<u>No.</u>	<u>Reagent</u>	<u>Conditions</u>	<u>Color</u>
1	Ammonium molybdate	10% in H ₂ SO ₄	Pink
2	Ammonium vanadate	10% in H ₂ SO ₄	Green
3	Original RPC reagent	?	Lavender
4	Dimethylaminobenzaldehyde	10% in acetic acid	Red
5	Sodium tungstate	1% in H ₂ SO ₄	Blue
6	Formaldehyde (40%)	10% in H ₂ SO ₄	Blue/Red
7	Uranyl nitrate	4% in H ₂ O	Red
8	Ferric chloride	4% in H ₂ O	Blue
9	Sodium arsenate	10% in H ₂ SO ₄	Blue
10	Sodium triphosphate	10% in H ₂ SO ₄	Violet
11	Benzidine	5% in H ₂ SO ₄	Yellow-Green
12	Stannous chloride	10% in H ₂ SO ₄	Red
13	Ammonium molybdate	10% in H ₃ PO ₄	Pink
14	Sodium molybdate	10% in H ₂ SO ₄	Pink
15	Sodium molybdate	10% in H ₃ PO ₄	Pink
16	Dimethylaminobenzaldehyde	10% in HCl	Green
17	Vanillin	50% in HCl	Violet
18	Sulfuric acid/nitric acid	1:1	Purple
19	Ferric chloride	1% in 1:1 H ₂ SO ₄ / acetic acid	Blue
20	Sodium nitrate	10% in H ₂ SO ₄	Blue-Violet
21	Potassium dichromate	1% in H ₂ SO ₄	Green
22	Nitric acid	Concentrated	Blue-Violet
23	Potassium chlorate	10% in H ₂ SO ₄	Green
24	Potassium perchlorate	10% in H ₂ SO ₄	Orange
25	Sulfuric acid/hydrochloric acid	1:1	Purple
26	Molybdic acid	10% in H ₂ SO ₄	Pink
27	Ammonium molybdate	10% in H ₂ SO ₄ + 10% potassium nitrate	Pink
28	Potassium perrhenate	1% in H ₂ SO ₄	Violet
29	Dimethylaminobenzaldehyde	10% in H ₂ SO ₄	Red
30	Potassium bromide	10% in H ₂ SO ₄	Green
31	Glyoxylic acid	10% in H ₂ SO ₄	Violet
32	Formaloxime	10% in H ₂ SO ₄	Blue
33	Piperonal	Sat. Sol. in 1/2 NH ₂ SO ₄	Red
34	Vanillin	10% in ethanol	Violet
35	Perchloric acid	conc.	Violet

TABLE I (Concluded)

<u>No.</u>	<u>Reagent</u>	<u>Conditions</u>	<u>Color</u>
36	Iodic acid/ammonium carbonate/ ferric chloride	1000:1000:1	Red
37	Iodic acid/ammonium carbonate/ ferric chloride/acetic acid	1000:1000:1:1000	Green
38	Sodium selenite	10% in H_2SO_4	Violet
39	Sodium selenite	10% in H_3PO_4	Violet
40	Diethyl oxalate	10% in H_2SO_4	?
41	Thiobarbituric acid	10% in H_2SO_4	?
42	Sodium nitrite	10% in 5% NaOH	Red
43	Manganese dioxide	10% in H_2SO_4	?
44	Sodium perborate	10% in H_2SO_4	?
45	Titanium tetrachloride	Neat	Red
46	Periodic acid	10% in H_2SO_4	?
47	Ceric Sulfate	10% in H_2SO_4	Orange
48	Ninhydrin	4 mg in 24 ml acetic acid + 30 ml H_2SO_4	Violet
49	Xanthhydrol	50 mg in 5 ml acetic acid mixed with 1 ml HCl + 14 ml acetic acid	?
50	Cobalt thiocyanate	10% in H_2O	?
51	Cobalt thiocyanate	10% in H_2SO_4	?
52	Dimethylaminobenzaldehyde	10% in $1NH_2SO_4$ in methanol	Red
53	Furfural	10% in H_2SO_4	Pink
54	Chloral	10% in H_2SO_4	Violet
55	Formaldehyde (40%)	10% in H_2SO_4 + 1% ferric chloride	Pink Blue
56	Formaldehyde (40%)	10% in H_2SO_4 + 10% ammonium molybdate	Pink
57	Formaldehyde (40%)	10% in H_2SO_4 + 10% uranyl nitrate	Red
58	Benzaldehyde	10% in ethanol	Orange
59	Urotropine	5% in H_2SO_4	Red
60	Iodine + iodide	1:1, 0.1% in 1N KOH	Green
61	Uranyl nitrate	10% in H_2SO_4	Red
62	Uranyl nitrate	10% in H_2O + 10% ferric chloride	Violet

TABLE II

COLOR REACTIONS OF MORPHINE AS TESTED
FOR THE PRESENTLY USED SYSTEM

No.	Color Reagent	Color Change Observed							
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine		Control	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
1	Ammonium molybdate, 10% in conc. H ₂ SO ₄ (colorless)	Dk. Purple	Green	Pink	-	-	-	-	-
2	Ammonium vanadate, 10% in conc. H ₂ SO ₄ (yellow)	-	-	-	-	-	-	-	-
3	Original RPC (colorless)	Deep Purple	Dk. Blue	Lavendar	Blue	Lt. Blue	Blue	Lt. Blue	Blue
4	Dimethylaminobenzaldehyde, 10% in conc. acetic acid (yellow)	-	-	-	-	-	-	-	-
5	Sodium tungstate, 1% in conc. H ₂ SO ₄ (colorless)	-	-	-	-	-	-	-	-
6	Formaldehyde (40%), 10% in conc. H ₂ SO ₄ (colorless)	Deep Red	Deep Red	Tan	Tan	-	-	-	-
7	Uranyl nitrate, 4% in H ₂ O (yellow)	-	-	-	-	-	-	-	-
8	Ferric chloride, 4% in H ₂ O (yellow)	-	-	-	-	-	-	-	-

TABLE II (Continued)

No.	Color Reagent	Color Change Observed							
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine		Control	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
9	Sodium arsenate, 10% in conc. H ₂ SO ₄ (colorless)	-	-	-	-	-	-	-	-
10	Sodium phosphate, 10% in conc. H ₂ SO ₄ (colorless)	-	-	-	-	-	-	-	-
11	Benzidine, 5% in conc. H ₂ SO ₄ (red)	-	-	-	-	-	-	-	-
12	Stannous chloride, 10% in conc. H ₂ SO ₄ (colorless)	-	-	-	-	-	-	-	-
13	Ammonium molybdate, 10% in conc. H ₃ PO ₄ (colorless)	Green	Green	Green	Green	Green	Green	Green	Green
14	Sodium molybdate, 10% in conc. H ₂ SO ₄ (colorless)	Dk. Purple	Dk. Blue	Purple	Blue	Lt. Blue	Blue	-	Blue
15	Sodium molybdate, 10% in conc. H ₃ PO ₄ (colorless)	-	-	-	-	-	-	-	-
16	Dimethylaminobenzaldehyde, 10% in conc. HCl (yellow)	-	Orange	-	-	-	-	-	-

TABLE II (Continued)

No.	Color Reagent	Color Change Observed							
		500 µg/ml Morphine Immediately	5 Min	5 µg/ml Morphine Immediately	5 Min	0.5 µg/ml Morphine Immediately	5 Min	Control Immediately	5 Min
17	Vanillin, 50% in conc. HCl								
18	Sulfuric acid/nitric acid, 1:1 (orange/yellow)	Orange	-	Orange	-	Orange	-	Orange	-
19	Ferric chloride, 1% in 1:1 H ₂ SO ₄ / acetic acid (yellow)	-	-	-	-	-	-	-	-
20	Sodium nitrate, 10% in conc. H ₂ SO ₄ (yellow)	Yellow/ Orange	Gold	-	-	-	-	-	-
21	Potassium-dichromate, 1% in conc. H ₂ SO ₄ (orange)	Green	Dk. Green	Green	Dk. Green	Green	Dk. Green	Green	Dk. Green
22	Nitric acid, conc. (colorless)	Red	Red	Red	Red	Red	Red	Red	Red
23	Potassium chlorate, 10% in conc. H ₂ SO ₄ (yellow)	-	-	-	-	-	-	-	-
24	Potassium perchlorate, 10% in conc. H ₂ SO ₄ (colorless)	Yellow	-	-	-	-	-	-	-
25	Sulfuric acid/ hydrochloric acid, 1:1 (colorless)	-	-	-	-	-	-	-	-

TABLE II (Continued)

No.	Color Reagent	Color Change Observed					
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
26	Molybdic acid, 10% in conc. H ₂ SO ₄ (colorless)	Dk. Purple	Dk. Blue	Lt. Purple	Blue	-	Blue
27	Ammonium molybdate, 10% in H ₂ SO ₄ and 10% potassium nitrate (colorless)	Green	Green	Green	Green	Green	Green
28	Potassium perchlenate, 1% in conc. H ₂ SO ₄ (faint violet)	Purple	Purple	Purple	Purple	Lt. Purple	Colorless
29	Dimethylaminobenzaldehyde, Red 10% in conc. H ₂ SO ₄ (orange)		Brown	Red	Red	Orange/ Red	Red
30	Potassium bromide, 10% in conc. H ₂ SO ₄ (orange)	Colorless	Colorless	Yellow	Yellow	Yellow	Yellow
31	Glyoxylic acid, 10% in conc. H ₂ SO ₄ (colorless)	Pink	Pink	Pink	Pink	Yellow	Yellow
32	Formaldehyde, 10% in conc. H ₂ SO ₄ (colorless)	-	-	-	-	-	-
33	Piperonal, sat. soln. in 1/2N H ₂ SO ₄ (colorless)	-	-	-	-	-	-

TABLE II (Continued)

No.	Color Reagent	Color Changes Observed							
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine		Control	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
34	Vanillin, 10% in ethanol (light yellow)	-	-	-	-	-	-	-	-
35	Perchloric acid conc. (colorless)	-	-	-	-	-	-	-	-
36	Iodic acid/ammonium carbonate/ferric chloride 1000:1000:1 (colorless solid)	-	-	-	-	-	-	-	-
37	Iodic acid/ammonium carbonate/ferric chloride/ acetic acid, 1000:1000:1:1000 (orange solid)	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
38	Sodium selenite, 10% in conc. H ₂ SO ₄ (light green)	Colorless	Orange	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
39	Sodium selenite, 10% in conc. H ₃ PO ₄ (light pink)	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
40	Diethyloxalate, 10% in conc. H ₂ SO ₄ (colorless)	-	Yellow	-	Yellow	-	Yellow	-	Yellow
41	Thiobarbituric acid, 10% in conc. H ₂ SO ₄ (yellow)	-	Green	-	Green	-	Green	-	Green
42	Sodium nitrite, 10% in 5% NaOH (colorless)	-	Yellow	-	Yellow	-	Yellow	-	Yellow

TABLE II (Continued)

No.	Color Reagent	Color Change Observed							
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine		Control	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
43	Manganese dioxide, 10% in conc. H_2SO_4 (dark blue)	Yellow	Brown	Yellow	Brown	Yellow	Brown	Yellow	Brown
44	Sodium perborate, 10% in conc. H_2SO_4 (colorless)	-	Yellow	-	-	-	-	-	-
45	Titanium tetrachloride	----- Unsuitable for Test -----							
46	Periodic acid, 10% in conc. H_2SO_4 (colorless)	Purple	Red	Red	Red	Brown	Red	Brown	Red
47	Ceric sulfate, 10% in conc. H_2SO_4 (orange)	-	Brown	-	Brown	-	Brown	-	Brown
48	Ninhydrin, 4 mg in 24 ml acetic and +30 ml H_2SO_4 (colorless)	-	-	-	-	-	-	-	-
49	Xanthhydrol, 50 mg in 5 ml acetic and mixed with 1 ml HCl + 14 ml acetic acid (brown)	Purple	Blue	Purple	Blue	Purple	Blue	Purple	Blue
50	Cobalt thiocyanate, 10% in water (purple)	-	-	-	-	Crimson	Crimson	Crimson	Crimson

TABLE II (Continued)

No.	Color Reagent	Color Change Observed							
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine		Control	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
51	Cobalt Thiocyanate, 10% in conc. H ₂ SO ₄ (pink)	Dk. Pink	-	-	-	-	-	-	-
52	Dimethylaminobenzaldehyde, 10% in H ₂ SO ₄ in methanol (yellow)	-	-	-	-	-	-	-	-
53	Furfural, 10% in conc. H ₂ SO ₄ (black)	Blue	Purple	Green	Purple	Green	Purple	Green	Purple
54	Chloral, 10% in H ₂ SO ₄ (colorless)	-	-	-	-	-	-	-	-
55	Formaldehyde (40%), 10% in conc. H ₂ SO ₄ + 1% ferric chloride (yellow)	-	-	-	-	-	-	-	-
56	Formaldehyde (40%), 10% in conc. H ₂ SO ₄ + 10% ammonium molybdate (blue)	Dk. Blue	-	-	-	-	-	-	-
57	Formaldehyde (40%), 10% in conc. H ₂ SO ₄ + 10% uranyl nitrate (yellow-green)	-	-	-	-	-	-	-	-

TABLE II (Continued)

No.	Color Reagent	Color Change Observed							
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine		Control	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
58	Benzaldehyde, 10% in ethanol (colorless)	-	-	-	-	-	-	-	-
59	Urotropine, 5% in conc. H ₂ SO ₄	----- Not Available Yet -----							
60	Iodine and iodine 1:1, 0.1% in 1N KOH (brown)	Yellow	Brown	Yellow	Brown	Yellow	Brown	Yellow	Brown
61	Uranyl nitrate, 10% in conc. H ₂ SO ₄ (yellow-green)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
62	Uranyl nitrate, 10% in water + 10% ferric chloride (orange)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
63	Phosphomolybdic acid, 10% in conc. H ₂ SO ₄ (yellow)	-	-	-	-	-	-	-	-
64	Sodium nitrate, 10% (colorless)	-	-	-	-	-	-	-	-

TABLE II (Continued)

No.	Color Reagent	Color Change Observed							
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine		Control	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
65	2(p-Iodophenyl)-3 (p-nitrophenyl)-5-phenyl tetrazolium chloride, 0.1% in methanol (pink)	Green	Green	Green	Green	Green	Green	Green	Green
66	m-Nitro neotetrazolium chloride, 0.1% in methanol (yellow)	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown
67	p-Tolyl tetrazolium red, 0.1% in methanol (yellow)	-	-	-	-	-	-	-	-
68	o-Tolyl tetrazolium red, 0.1% in methanol (red)	-	-	Dk. Red	-	Dk. Red	-	-	-
69	Tetrazolium blue (diformazan), 0.1% in methanol (purple)	-	-	-	-	-	-	-	-
70	Tetrazolium blue (BT), 0.1% in methanol (yellow)	-	Pink	-	Pink	-	Pink	-	Pink

TABLE II (Continued)

No.	Color Reagent	Color Change Observed							
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine		Control	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
71	Fast red B, 0.25% in 0.1N HCl (yellow)	Brown	Green	Brown	Green	Brown	Green	Green	Green
72	Fast violet B, 0.25% in 0.1N HCl (yellow)	-	-	-	-	-	-	-	-
73	Fast scarlet 9, 0.25% in 0.1N HCl (brown)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
74	Fast yellow GC, 0.25% in 0.1N HCl (yellow)	-	-	-	-	-	-	-	-
75	Fast bordeaux 3B, 0.25% in 0.1N HCl (colorless)	Yellow	-	Yellow	-	Yellow	-	Yellow	-
76	Azo-o-toluidine, 0.25% in 0.1N HCl (orange)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
77	Fast red AL, 0.25% in 0.1N HCl (brown)	Orange	Orange	Orange	Orange	Orange	Orange	Orange	Orange

TABLE II (Continued)

No.	Color Reagent	Color Change Observed							
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine		Control	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
78	Fast red TR, 0.25% in 0.1N HCl (colorless)	-	Yellow	-	Yellow	-	Lt. Yellow	-	Lt. Yellow
79	Fast blue BB, 0.25% in 0.1N HCl (brown)	Pink	Green	Pink	Green	Pink	Green	Pink	Green
80	Fast scarlet 2G, 0.25% in 0.1N HCl (brown)	Orange	Orange	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
81	Naphthanil diazo scarlet, 0.25% in 0.1N HCl (red)	-	-	-	-	-	-	-	-
82	o-Nitro-aniline-diazotate, 0.25% in 0.1N HCl (yellow)	-	-	-	-	-	-	-	-
83	Dichloroquinone chloride, 0.25% in 0.1N HCl (brown)	Pink	-	Pink	-	Pink	-	Pink	-
84	Molybdic anhydride, 10% in conc. H ₂ SO ₄ (colorless)	Purple	Blue	-	Green	-	Green	-	Green

TABLE II (Continued)

No.	Color Reagent	Color Change Observed					
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
85	Reagent No. 1, with 1% NaCl (yellow)	Purple	Blue	-	Blue	-	Blue
86	Reagent No. 1, with 0.1% FeCl ₃ (yellow)	Purple	Blue	Blue	Blue	-	Blue
87	Reagent No. 1, with 1% NaNO ₃ (yellow)	Blue	Blue	Blue	Blue	Blue	Blue
88	Reagent No. 1, with 1% NaNO ₂ (green)	Blue	Blue	Blue	Blue	Blue	Blue
89	Reagent No. 1, with 1% Na ₂ SeO ₃ (green)	Purple	Blue	Lt. Purple	Blue	Lt. Blue	Blue
90	Reagent No. 1, with 1% SnCl ₂ (blue)	Blue	Blue	Blue	Blue	Blue	Blue
91	Reagent No. 14, with 1% NaCl (green)	Dk. Purple	Blue	Lt. Purple	Blue	Lt. Blue	Blue
						Lt. Blue	Blue

TABLE II (Continued)

No.	Color Reagent	Color Change Observed					
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
92	Reagent No. 14, with 0.1% FeCl ₃ (green)	Dk. Blue	Blue	Colorless	Blue	Colorless	Blue
93	Reagent No. 14, with 1% NaNO ₃ (green)	Blue	Blue	Blue	Blue	Blue	Blue
94	Reagent No. 14, with 1% NaNO ₂ (green)	Blue	Blue	Blue	Blue	Blue	Blue
95	Reagent No. 14, with 1% Na ₂ SeO ₃ (green)	Purple	Blue	Blue	Blue	Blue	Blue
96	Reagent No. 14, with 1% SnCl ₂ (green)	Blue	Blue	Blue	Blue	Blue	Blue
97	Reagent No. 26, with 1% NaCl (green)	Purple	Blue	Lt. Purple	Blue	Blue	Blue
98	Reagent No. 26, with 0.1% FeCl ₃ (green)	Purple	Blue	Lt. Purple	Blue	Blue	Blue

TABLE II (Continued)

No.	Color Reagent	Color Change Observed							
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine		Control	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
99	Reagent No. 26, with 1% NaNO_3 (green)	Turquoise	Turquoise	Turquoise	Turquoise	Turquoise	Turquoise	Turquoise	Turquoise
100	Reagent No. 26, with 1% NaNO_2 (green)	Turquoise	Turquoise	Turquoise	Turquoise	Turquoise	Turquoise	Turquoise	Turquoise
101	Reagent No. 26, with 1% Na_2SeO_3 (green)	Purple	Blue	Lt. Purple	Blue	Blue	Blue	Lt. Blue	Blue
102	Reagent No. 26, with 1% SnCl_2 (blue)	-	-	-	-	-	-	-	-
103	Reagent No. 84, with 1% NaCl (colorless)	Purple	Green	-	Green	-	Green	-	Green
104	Reagent No. 84, with 0.1% FeCl_3 (colorless)	Purple	Green	-	Green	-	Green	-	Green
105	Reagent No. 84, with 1% NaNO_3 (yellow)	Green	Green	Green	Green	Green	Green	Green	Green

TABLE II (Concluded)

No.	Color Reagent	Color Change Observed							
		500 µg/ml Morphine		5 µg/ml Morphine		0.5 µg/ml Morphine		Control	
		Immediately	5 Min	Immediately	5 Min	Immediately	5 Min	Immediately	5 Min
106	Reagent No. 84, with 1% NaNO ₂ (gray)	Green	Green	Green	Green	Green	Green	Green	Green
107	Reagent No. 84, with 1% Na ₂ SeO ₃ (brown)	Green	Green	Green	Green	Green	Green	Green	Green
108	Reagent No. 84, with 1% SnCl ₂ (green)	Gray	Gray	Gray	Gray	Gray	Gray	Gray	Gray
109	Titanium oxide, 0.5% in conc. H ₂ SO ₄ (gray)	Purple	Beige	-	-	-	-	-	Yellow
110	Reagent No. 1, with 1% iodic acid (yellow)	Purple	Blue	Pink	Blue	Pink	Blue	-	Gray
111	Reagent No. 14, with 1% iodic acid (yellow)	Black	Blue	Beige	Blue	-	Blue	-	Blue
112	Reagent No. 14, with 1% selenium dioxide (colorless)	Dk. Purple	Dk. Blue	Purple	Blue/Green	-	Blue	-	Blue
113	Reagent No. 14, with 0.1% selenium dioxide (colorless)	Dk. Purple	Dk. Blue	Purple	Green	-	Blue	-	Blue

Blank (-) indicates no change in color.

TABLE III

COLOR REACTIONS OF POSSIBLE INTERFERENCES

<u>Drug</u>	<u>Color Formation</u>			
	<u>10 µg/ml Drug in Urine</u>		<u>5 µg/ml Drug in Urine</u>	
	<u>Immediately</u>	<u>5 Min</u>	<u>Immediately</u>	<u>5 Min</u>
1 Morphine	Purple	Tan	Medium Purple	Beige
2 Codeine	Gray	Blue	Yellow	Blue
3 Paragoric	Light Purple	Blue	Light Purple	Blue
4 Heroin	Purple	Gray	Light Purple	Beige
5 Dilaudid	Gray	Blue	Yellow	Blue
6 Cocaine	Gray	Blue	Yellow	Blue
7 Demerol	Pink	Gray	Yellow	Gray
8 Quinine	Gray	Blue	Blue/Gray	Blue
9 Nicotine	Gray	Gray	Blue/Gray	Blue
10 Aspirin	Gray	Blue	Yellow	Blue
11 Phenobarbital	Yellow	Gray	Yellow	Blue
12 Amphetamine	Yellow	Tan	Yellow	Blue
13 Methamphetamine	Gray	Blue	Yellow	Blue
14 Caffeine	Yellow	Blue	Yellow	Blue
15 Methadone	Gray	Blue	Yellow	Blue
16 Meproamate	Yellow	Blue	Yellow	Blue
17 Ritalin	Yellow	Gray	Yellow	Gray
18 Phencyclidine	Yellow	Gray	Yellow	Blue
19 Librium	Yellow	Beige	Yellow	Blue
20 Lobeline	Yellow	Tan	Yellow	Blue
21 Tofranil	Yellow	Gray	Yellow	Blue

TABLE IV

RESULTS OF EXTRACTION BY SELECTED SOLVENTS

	Solvent	Color Formation			
		5 µg/ml Morphine in Urine		Control	
		Immediately	5 Min	Immediately	5 Min
1	Original solvent	Purple	Blue	Yellow	Gray
2	(30% isopropanol in chloroform)	Weak Purple	Yellow	Yellow	Yellow
3	Chloroform	Yellow	Blue	Yellow	Blue
4	Diethyl ether	Blue	Blue	Blue	Blue
5	Butyl ether	--*	Yellow	--	Yellow
6	1:2-Dichloroethane	--	Blue	--	Blue
7	Ethyl acetate	Blue	Blue	Blue	Blue
8	tert-Butanol	Blue	Blue	Blue	Blue
9	sec-Butanol	Blue	Blue	Blue	Blue
10	iso-Butanol	Blue	Blue	Blue	Blue
11	Amyl alcohol	Yellow	Yellow	Yellow	Yellow
12	Methylene chloride	Yellow	Blue	Yellow	Yellow
13	Carbon tetrachloride	Pink	Beige	Yellow	Yellow
14	1:2-Dichloroethane, 3% in chloroform	--	Beige	--	Beige
15	1:2-Dichloroethane, 10% in chloroform	--	Beige	--	Beige
16	1:2-Dichloroethane, 20% in chloroform	--	Beige	--	Beige
17	1:2-Dichloroethane, 30% in chloroform	--	Beige	--	Beige
18	1:2-Dichloroethane, 50% in chloroform	--	Beige	--	Beige
19	1:2-Dichloroethane, 70% in chloroform	--	Beige	--	Beige
20	1:2-Dichloroethane, 90% in chloroform	--	Beige	--	Beige
21	Ethyl acetate, 3% in chloroform	Pink	Beige	Yellow	Beige
22	Ethyl acetate, 10% in chloroform	Pink	Beige	Yellow	Beige
23	Ethyl acetate, 20% in chloroform	Pink	Beige	Yellow	Beige
24	Ethyl acetate, 30% in chloroform	Pink	Beige	Yellow	Beige
25	Ethyl acetate, 50% in chloroform	--	Blue	--	--
26	Ethyl acetate, 70% in chloroform	--	Blue	--	Blue
27	Ethyl acetate, 90% in chloroform	--	Blue	--	Blue

TABLE IV (Continued)

	Solvent	Color Formation			
		5 µg/ml Morphine in Urine		Control	
		Immediately	5 Min	Immediately	5 Min
27	tert-Butanol, 3% in chloroform	Blue	Blue	Blue	Blue
28	tert-Butanol, 10% in chloroform	Blue	Blue	Blue	Blue
29	tert-Butanol, 20% in chloroform	Blue	Blue	Blue	Blue
30	tert-Butanol, 30% in chloroform	Blue	Blue	Blue	Blue
31	tert-Butanol, 50% in chloroform	Blue	Blue	Blue	Blue
32	tert-Butanol, 70% in chloroform	Blue	Blue	Blue	Blue
33	tert-Butanol, 90% in chloroform	Blue	Blue	Blue	Blue
34	sec-Butanol, 3% in chloroform	--	Blue	--	Blue
35	sec-Butanol, 10% in chloroform	--	Blue	--	Blue
36	sec-Butanol, 20% in chloroform	Light Purple	Blue	--	Blue
37	sec-Butanol, 30% in chloroform	Yellow	Blue	Yellow	Blue
38	sec-Butanol, 50% in chloroform	Yellow	Blue	Yellow	Blue
39	sec-Butanol, 70% in chloroform	Blue	Blue	Blue	Blue
40	sec-Butanol, 90% in chloroform	Blue	Blue	Blue	Blue
41	iso-Butanol, 3% in chloroform	Light Purple	Blue	Yellow	Blue
42	iso-Butanol, 10% in chloroform	Light Purple	Blue	Yellow	Blue
43	iso-Butanol, 20% in chloroform	Light Purple	Blue	Yellow	Blue
44	iso-Butanol, 30% in chloroform	Light Purple	Blue	Yellow	Blue
45	iso-Butanol, 50% in chloroform	Light Purple	Blue	Blue	Blue
46	iso-Butanol, 70% in chloroform	Blue	Blue	Gray	Blue
47	iso-Butanol, 90% in chloroform	Gray	Blue	Blue	Blue
48	Amyl alcohol, 3% in chloroform	Blue	Blue	Blue	Blue
49	Amyl alcohol, 10% in chloroform	Light Purple	Blue	Yellow	Blue
50	Amyl alcohol, 20% in chloroform	Light Purple	Blue	Yellow	Blue
51	Amyl alcohol, 30% in chloroform	Light Purple	Blue	Yellow	Blue
52	Amyl alcohol, 50% in chloroform	Light Purple	Blue	Yellow	Blue
53	Amyl alcohol, 70% in chloroform	Light Purple	Blue	Yellow	Blue
54	Amyl alcohol, 90% in chloroform	Blue	Blue	Blue	Blue

TABLE IV (Concluded)

	Solvent	Color Formation			
		5 µg/ml Morphine in Urine		Control	
		Immediately	5 Min	Immediately	5 Min
55	Methylene chloride, 3% in chloroform	Light Purple	Beige	Yellow	Beige
56	Methylene chloride, 10% in chloroform	Light Purple	Beige	Yellow	Beige
57	Methylene chloride, 20% in chloroform	Light Purple	Beige	Yellow	Beige
58	Methylene chloride, 30% in chloroform	Light Purple	Beige	Yellow	Beige
59	Methylene chloride, 50% in chloroform	Light Pink	Gray	Yellow	Gray
60	Methylene chloride, 70% in chloroform	Yellow	Gray	Yellow	Gray
61	Methylene chloride, 90% in chloroform	Yellow	Gray	Yellow	Gray
62	iso-Propanol, 3% in chloroform	Light Purple	Beige	Gray	Beige
63	iso-Propanol, 10% in chloroform	Light Purple	Gray	Gray	Gray
64	iso-Propanol, 15% in chloroform	Light Purple	Blue	Gray	Gray
65	iso-Propanol, 20% in chloroform	Medium Purple	Blue	Gray	Gray
66	iso-Propanol, 25% in chloroform	Medium Purple	Blue	Gray	Blue
67	iso-Propanol, 30% in chloroform	Purple	Blue	Gray	Blue/Gray
68	iso-Propanol, 35% in chloroform	Purple	Blue	Yellow/Gray	Gray
69	iso-Propanol, 40% in chloroform	Medium Purple	Blue	Gray	Blue
70	iso-Propanol, 50% in chloroform	Green/Blue	Blue	Gray/Blue	Blue
71	iso-Propanol, 70% in chloroform	Green/Blue	Blue	Green/Blue	Blue
72	iso-Propanol, 90% in chloroform	Blue	Blue	Blue	Blue

* Note - (-) indicates no color formation.

TABLE V

RESULTS OF COLOR TEST FOR SPIKED
MORPHINE IN URINE

<u>Concentration of Spiked Morphine ($\mu\text{g/ml}$)</u>	<u>Number of Experiments</u>	<u>Number of Positives</u>	<u>Number of Negatives</u>	<u>Percent Correct</u>
Blank	11	0	11	100
3	9	9	0	100
2	36	36	0	100
1-1/3	7	7	0	100
1	50	47	3	94
2/3	28	9	19	32
1/2	<u>66</u>	<u>26</u>	<u>40</u>	<u>39</u>
<u>Total</u>	207	134	73	70

TABLE VI

RESULTS OF COLOR TEST FOR USER
URINE FROM MORPHINE PATIENTS

<u>Date</u> <u>1972</u>	<u>Patient</u>	<u>Concentration</u> <u>of Morphine^{a/}</u>	<u>Color Test Results</u>	
			<u>Positive Observations/Total Observations</u>	
4/13	D	7.00	8/8	+
4/10	C	5.90	4/8	?
4/12	A	5.60	8/8	+
4/17	C	4.35	2/8	-
4/12	A	4.15	1/8	-
4/12	D	4.15	4/8	?
5/1	F	3.90	6/8	+
4/15	C	3.65	5/8	+
4/26	F	3.65	6/8	+
4/11	C	3.30	0/8	-
4/14	A	3.30	3/8	-
4/25	F	3.00	3/8	-
5/1	E	2.90	6/8	+
4/11	A	2.60	1/8	-
4/24	F	2.50	3/8	-
4/13	C	2.15	2/8	-
4/16	C	1.95	1/8	-
4/15	D	1.85	8/8	+
4/12	C	1.75	1/8	-
4/16	C	1.25	0/8	-
4/12	C	1.05	0/8	-
4/29	F	1.05	5/8	+
4/30	F	1.05	6/8	+
4/12	D	1.00	0/8	-
4/10	A	0.80	0/8	-
4/11	D	0.80	0/8	-
4/14	C	0.80	0/8	-
4/24	F	0.75	1/8	-
4/15	C	0.75	1/8	-
4/11	C	0.60	0/8	-
4/16	A	0.50	1/8	-
4/15	A	0.40	0/8	-
4/17	B	0.10	4/8	?
4/11	D	< 0.10	1/8	-
4/14	C	< 0.10	0/8	-
4/15	D	< 0.10	3/8	-
4/12	C	< 0.10	2/8	-
Pool No. 1		1.07	75/80	+
Pool No. 2		2.15	80/80	+
Pool No. 3		2.35	80/80	+
Pool No. 4		1.55	8/8	+

^{a/} Determined fluorimetrically.

VII. BIBLIOGRAPHY

1. Aloy, M. J., "Characterisation de la morphine et des phenols a l'aide des sels d'uranium," Bull. Societe Chim., (4), 15, 680 (1914).
2. Aloyd, M. J., "Precipitation de quelques alcaloides par le nitrate d'uranium. Reaction de la morphine," Bull. Soc. Chim., (3), 29, 610 (1903).
3. Babko, A. K., and V. S. Konyushko (Inst. Gen. and Inorg. Chem., Kiev), "Methods for Extractive-Photometric Determination of Alkaloids. I. Acid-Base Properties of the Components and the Effect of pH on the Extraction of Alkaloid Compounds with Acid Dyes," Zh. Analit. Khim., 29(4), 486-90 (1966) (Russ.).
4. Bentley, Kenneth Walter, "The Chemistry of the Morphine Alkaloids," Oxford, Clarendon Press, 1954.
5. Blackmore, D. J., A. S. Curry, T. S. Hayes, and E. R. Rutter, "Automated Analysis for Drugs in Urine," Clin. Chem., 17(9), 896-802 (1971) (Eng.)
6. Bronciner, A. L., "Als Reagentien zum Nachweis einiger Alkaloide verwez," Z. Anal. Chem., 37, 62 (1898).
7. "Narcotic Drugs: Biochemical Pharmacology," Edited by Doies H. Clouet pp. 40-46, Plenum Press, New York, (1971).
8. Couerbe, J. P., "Analyses de plusieurs Principes immediats. Action de l'Acide Nitrique et sulfurique ser quelque substances organiques. Preuve evident que l'Formule de l'Ether isole 'est differente de cel de l'Ether en Combinaison," Ann. Chim. Phys., 59 (2), 136 (1835).
9. Czapski, A. and W. Fresenius, "Auf Gerichtliche Chemie Bezugliche," Z. Anal. Chem., 38, 466 (1899).
10. Dittmar, M., "Mittheilungen" "Ueber die Reaction zwischen Chlorjod und den Alkaloiden," Ber., 18, 1612 (1885).
11. Donath, Dr. J., "Zwei Morphininreactionen," J. Pr. Chem., 33 (2), 563 (1886).
12. Duflos, As, "Beitrage zur chemischen Kenntnifs des Morphins und Narkotins," Pharm. Zentralhalle, 6, 105 (1885)
13. Dutkiewica, T., and I. Lisikowa, "Colorimetric Determination of Morphine," Acta Poton. Pharm., 19, 149-55 (1962) (Polish).
14. Ehrlich-Rogozinski, S., and Nicholas D. Cheronis, "The Identification and Determination of Morphine," Micro Chemical Journal, 7, 336-56 (1963).

15. Eiloart, A., "Reactions of Quinine, Narcotine, and Morphine with Bromine," Chemical News, 50, 102 (1884).
16. Elliott, Henry W., Norman Nomof, Kenneth Parker, Marjorie L. Dewey, and E. Leong Way, "Comparison of the Nalorphine Test and Urinary Analysis in the Detection of Narcotic Use," Clin. Pharmacol. Therap., 5(4), 405-13 (1964 (Eng.)).
17. Fraude, Georg, "Ueberchlorsaure, ein neues Reagenz auf Alkaloide," Ber., 12, 1558 (1879).
18. Fresenius, H., "Chemische Analyse Organischer Korper," Z. Anal. Chem., 19, 355 (1880).
19. Fulton, Charles C., "Sulformorphid, and the Purple Fluorescence Test, A New Drivative Test for Morphine," J. Am. Pharm. Assoc., 26, 726 (1937).
20. Itallie, L. Van, and A. J. Steenhauer, "Vanillan und Piperonal Als Reagenzien auf Alkaloide," Arch. Pharm., 265, 696 (1928).
21. Leake, Chauncey D., "Iodoxybenzoate as a Test Reagent for Free Phenolic Hydroxyl Groups in Organic Compounds," Proc. Soc. Exptl. Biol. Med., 28, 148 (1930).
22. Lefort, M. J., "Etudes Chimiques et Toxicologiques sur la morphine suivies d'observations sur son passage dans l'economie animale," J. Pharm., 40(3), 97 (1861).
23. Levi, Leo, "Morphine-Marine Complex," Anal. Chem., 29, 470-4 (1957).
24. Mesnard, P., G. Le Roux, M. Bertucat, and J. Marzat, "Mechanism of Denige's Reaction with Morphine," Pharm. Acta Helv., 37, 440-51 (1962).
25. Nadler, M. G., "Sur un nouvel alcaloide derive," de la morphine," Bull. Societe Chem., (2), 21, 326 (1874).
26. Neubauer, C., "Chemische Analyse Organischer Korper," Z. Anal. Chem., 13, 235 (1874).
27. Parker, Kenneth D., M. Crim, C. H. Hine, N. Nomof, and H. W. Elliott, "Urine-Screening Techniques Employed in the Detection of Users of Narcotics and Their Correlation with the Nalorphine Test," J. Forensic Sci., 11(2), 152-66 (1966) (Eng.).

28. Pesez, M. M., "Sur deux noreaux reactifs differentiels de la morphine et de l'oxy dimorphine," J. Pharm. Chem., 27, 255 (1938).
29. Pesez, M. M., "Sur une nouvelle reaction chromatique de la morphine at des alcaloides derives," J. Pharm. Chim., 25, 504 (1937).
30. Pollack, Benjamin, "The Validity of the Forrest Reagent Test for the Detection of Chlorpromazine or Other Phenothiazines in the Urine," Am. J. Psychiat., 115, 77-8 (1958), cf. Forrest and Forrest, Am. J. Psychiat., 113, 931 (1957).
31. Reichard, C., "Ueber eine neve Reaction zum Nachweise des Morphins," Z. Anal. Chem., 42, 95 (1903).
32. Reichard, Von C., "Beitrage zur Kenntnis uer Alkaloidreaktionen," Chemiker-Zeitung, 28, 1102 (1904).
33. Sakurai, Hiroshi, "Analyses of Opium Alkaloids. VII. New Color Reaction of Morphine and Its Application to Colorimetric Determination," Yakugaku Zasshi Pharm. Soc. of Japan, 81, 155-59 (1961).
34. Schaer, Ed, "Ueber die Einwirkung des Morphins, sowie des Acetanilids auf Mischungen von Ferrisalz und Kalimferricyanid," Arch. Pharm., 234, 348 (1896).
35. Schieser, David W., "Free Radicals in Alkaloidal Color Identification Tests," J. Pharm. Sci., 53, 909-13 (1964).
36. Siebold, "A New Test for Morphia," Am. J. Pharm., 3(4), 544 (1873).
37. Splies, Robert G., and James M. Shellow, "Color Reactions of Morphine Derivatives," J. Chem. Eng. Data, 11(1), 123-4 (1966).
38. Takemori, A. E., "An Ultrasensitive Method for the Determination of Morphine and Its Application in Experiments In Vitro and In Vivo," Biochem. Pharmacol, 17(8), 1627-35 (1968) (Eng.).
39. Tattersall, T., "Notes on the Alkaloids," Chemical News, 41, 63 (1880).
40. Theil and Pelletier, "Neue Versuche uber Opium," Ann., 5, 150 (1833).
41. "Zum Nachweis Von Morphin," Z. Anal. Chem., 5, 214 (1866).
42. "Nuova reasione della marfina," Gazetta Chimica, Italiana, Rome, 7, 297 (1877).

43. Vegh, Antal, Antal Brantner, Gyorgy Szasz, Zs. Budvari, and K. Gracza, "Identification of Powder Mixtures. I. The Detection of Morphine Ethylmorphine, and Codeine," Acta Pharm. Hung., 33, 57-66 (1963) (Hungarian).
44. Vulpius, G., "Eine Morphinreaction," Arch. Pharm., 225, 256 (1887).
45. Weppen, Dr. Herm, "Zum Nachweise von Veratrin und Morphin," Arch. Pharm., 205, 112 (1871).
46. Woker, G., and I. Antener, "Neue Reaktionen der Ascorbinsaure ein Beitrag zur Kenntniss der Farbreaktionen von Alkaloiden and Sterinen," Helv. Chim. Acta, 21, 1345 (1938).

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13. ABSTRACT <p>This report details work conducted on a project designed to improve the sensitivity of a field test kit for detecting morphine in urine. Color reagents, extraction parameters and techniques were evaluated to improve the sensitivity to less than 1 ug/ml morphine in urine. Final evaluation of the redesigned field kit using spiked urine indicated the project was successful; however, evaluation with user urine was inconclusive due to the shortage of a supply of fresh user urine.</p>			

14.

KEY WORDS

LINK A

LINK B

LINK C

ROLE

WT

ROLE

WT

ROLE

WT

Urine

Morphine

Heroin

Reagent

Solvent

Alcohol

Chloroform

Sulfuric Acid

Sodium Carbonate

Sodium Bromide